Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Saliva extraction-free SARS-CoV-2 RT-PCR

The Nebraska Extraction-free Saliva (NEfS) assay used for this program was optimized for high-throughput testing with considerations for minimizing overall testing costs, overcoming potential shortages of reagents, and decreasing turnaround time. In brief, 50 µL of well-mixed saliva is added to 6.3 µL of proteinase K (New England Biolabs, P8107S), then shaken and heated at 2,200 RPM and 95ºC for 5 minutes. Five microliters of the sample preparation are added to 15 µL of PCR master mix (TaqPath 1-Step RT-pPCR Master Mix, ThermoFisher Scientific A15299) containing primers and probes for detection of SARS-CoV-2 nucleoprotein RNA and human RNaseP RNA (Integrated DNA Technologies, IDT). RT-PCR is performed on QuantStudio7 Pro thermocyclers (ThermoFisher Scientific).

Wastewater processing and SARS-CoV-2 RT-PCR

For RT-PCR testing, samples were processed using the Qiagen RNeasy PowerSoil Total RNA kit (Qiagen, 12866-25) following the manufacturer's instructions. For each sample, 70 mL of well-mixed wastewater was divided into two 50 mL conical tubes and centrifuged at 3,500 x g for 20 minutes to concentrate solids within the specimen. The supernatant was carefully removed, and the remaining pellet was resuspended in up to 2 mL of water and used as input for the PowerSoil extraction. VetMAX™ Xeno™ Internal Positive Control RNA (ThermoFisher Scientific, A29761) was spiked into each sample to verify extraction efficiency. SARS-CoV-2 was detected using the IDT 2019-nCoV RUO kit (IDT, 10006713). A standard curve was generated using a dilution series of quantitative synthetic SARS-CoV-2 RNA (ATCC, VR-3276SD). For total and suspended solid analysis, wastewater samples were processed following ASTM D5907-18.

Air and surface sample collection and processing for SARS-CoV-2 RT-PCR

For air samples, cartridges were removed from the sampler and placed in a sealed bag for transport. Surface samples were collected using a prewetted (3 mL of sterile PBS) cotton swipe by wiping in an S-pattern, in two directions, over an approximately 100 cm^2 area. Following collection, surface swipes were placed into a 50 mL conical tub for transport. All air and surface samples were transported on ice to UNMC for processing. For air samples, the two metallic probes were removed from each cartridge and placed in a 15 mL conical tube with 10 mL PBS and then shaken by hand for one minute to liberate collected particles. Surface samples were recovered by adding 10 mL of PBS to the 50 mL conical tube and hand shaking for one minute. Following recovery, 400 µL of each recovered sample was extracted using the EZ1 Advanced XL Extractor (Qiagen, Hilden, Germany) with the EZ1® Virus Mini Kit v2.0.

SARS-CoV-2 whole-genome sequencing

RNA was extracted from saliva samples on a KingFisher Flex (ThermoFisher Scientific) using the MagMax Viral/Pathogen II (MVP II) Nucleic Acid Isolation kit (ThermoFisher Scientific, A48383) following the manufacturer's instructions for processing saliva samples. Samples were processed for sequencing by two different methods. For Oxford Nanopore Technologies (ONT) sequencing, the ARTIC multiplex PCR method V3 primer set was used following the publicly available protocol for amplification, library preparation, and analysis.[14] For Illumina-based sequencing, we used Swift Biosciences Normalase Amplicon SARS-CoV-2 V1 Panel[15] following the manufacturer's instructions. Libraries were sequenced on a 2 x 151 bp iSeq100 run. Genomes were assembled using the TAYLOR pipeline and consensus genomes were submitted to GISAID (EPI_ISL_1016947 – EPI_ISL_1016967).

The genomes from our study were aligned with 177 other genomes from Nebraska state, available on the GISAID repository (contributor acknowledgments provided in eTable 3) with MAFFT v1.4.0, as implemented in Geneious Prime v2021.0.3. A phylogenetic tree was inferred using RAxML v4.0 under the GTR+GAMMA model.

Statistical analyses

Data handling and analyses were performed in Microsoft Excel® and SAS®. Additional graphics were generated in GraphPad Prism 9®. In addition to descriptive epidemiology, each metadata element was assessed in univariate, and when appropriate, bivariate analyses for assessing impact on participants' first positive result. When statistically significant non-identity estimates of effect were identified or for presumed confounders that could be reasonably characterized, those elements were advanced to multivariate regression analysis. Community case rate data was not sufficiently granular to allow transforming zipcodes to levels of background COVID-19 risk. Socioeconomic status

of involved zipcodes was homogenous. To limit participation effects while still exploring differential community impacts, student zip codes of residence were grouped by more to less common among students. Staff zip codes were grouped by geographic quadrant centered in downtown Omaha.

eTable 1. Acknowledgment of Contributors to GISAID SARS-CoV-2 Genome Sequences Used in This Study

We gratefully acknowledge the following Authors from the Originating laboratories responsible for obtaining the specimens, as well as the Submitting laboratories where the genome data were generated and shared via GISAID, on which this research is based.
All Submitters of data may be contacted directly via www.gisaid.org
Authors are sorted alphabetically.

Genome entries	Originating laboratory	Submitting laboratory	Contributors
EPI_ISL_424874 EPI_ISL_424875	NE Public Health Laboratory	Pathogen Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Yan Li, Krista Queen, Clinton R. Paden, Rachel Marine, Anna Uehara, Ying Tao, Jing Zhang, Haibin Wang, Mary S. Keckler, Alison S. Laufer Halpin, Christopher A. Elkins, Suxiang Tong
EPI_ISL_677521	University of Wisconsin-Madison AIDS Vaccine Research Laboratories	University of Wisconsin-Madison AIDS Vaccine Research Laboratories	Gage Moreno, Katarina Braun, et al. AIDS Vaccine Research Laboratories
EPI_ISL_751563 EPI_ISL_751583 EPI_ISL_751585 EPI_ISL_751608 EPI_ISL_751638 EPI_ISL_751681 EPI_ISL_751732 EPI_ISL_751734	NE Public Health Laboratory	Genomics and Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Krista Queen, Yan Li, Ying Tao, Jing Zhang, Anna Uehara, Anna Montmayeur, Clinton R. Paden, Peter W. Cook,Rachel Marine, Mili Sheth, Haibin Wang, Justin Lee, Suxiang Tong
EPI_ISL_756369 EPI_ISL_756370 EPI_ISL_756371 EPI_ISL_756372 EPI_ISL_756373 EPI_ISL_756374	CUMC - CHI Bergan Mercy	Creighton University School of Medicine, Departments of Medical Microbiology and Pharmacology and Neuroscience	Michael Belshan, Morgan A. Raine, Anne V. Cheng, Christopher J. Destache, Richard V. Goering, Jacob A. Siedlik, Holly A. Stessman
EPI_ISL_812532 EPI_ISL_812550 EPI_ISL_812571 EPI_ISL_812572 EPI_ISL_812605 EPI_ISL_812627 EPI_ISL_812655 EPI_ISL_812676 EPI_ISL_812695 EPI_ISL_812705	United States Air Force School of Aerospace Medicine	United States Air Force School of Aerospace Medicine	Anthony Fries, Jennifer Meyer, Amanda Javorina, Sarah Purves, William Gruner, Clarise Starr, Elizabeth Macias

EPI_ISL_831742 EPI_ISL_831744 EPI_ISL_831754 EPI_ISL_831819 EPI_ISL_831825 EPI_ISL_831834 EPI_ISL_831841 EPI_ISL_831857 EPI_ISL_831866 EPI_ISL_831887	United States Air Force School of Aerospace Medicine	United States Air Force School of Aerospace Medicine	Anthony Fries, Jennifer Meyer, William Gruner, Amanda Javorina, Sarah Purves, Clarise Starr, Elizabeth Macias
EPI_ISL_850933	Helix / Illumina	Genomics and Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Peter W. Cook, Dhwani Batra, Ben L. Rambo-Martin Eileen de Feo, Jan Antico, Christine Tran, Matthew Tolentino, Shannon Wickline, Kim Gietzen, Brad Sickler, Jingtao Liu, Eric Allen, Phil Febbo, Summer Galloway, Nicole L. Washington, Simon White, Geraint Levan, Kelly Schiabor Barrett, Elizabeth Cirulli, Alexandre Bolze, Ary Ascencio, Charlotte Rivera-Garcia, Ryan Cho, Jason Nguyen, Sherry Wang, Jimmy Ramirez, Tyler Cassens, Efren Sandoval, Magnus Isaksson, William Lee, David Becker, Marc Laurent, James Lu, Clinton R. Paden, Suxiang Tong, Duncan MacCannell
EPI_ISL_876873	Quest Diagnostics	Quest Diagnostics	Rosenthal,S.H., Gerasimova,A., Kagan,R.M., Anderson, B., Hua, M., Liu Y., Bernstein, L.E., Livingston, K.E., Perez, A., Shalhout, D.F., Shlyakhter, I.A., Owen, R., Tanpaiboon, P., Lacbawan, F.
EPI_ISL_884375	Infectious Diseases, Quest Diagnostics	Infectious Diseases, Quest Diagnostics	Rosenthal,S.H., Gerasimova,A., Kagan,R.M., Anderson,B., Bernstein,L.E., Livingston,K.E., Hua,M., Liu,Y., Shalhout,D.F., Owen,R., Lacbawan,F.
EPI_ISL_886189 EPI_ISL_886258 EPI_ISL_886278 EPI_ISL_886334 EPI_ISL_886376 EPI_ISL_886377 EPI_ISL_886430 EPI_ISL_886530 EPI_ISL_886544 EPI_ISL_886596 EPI_ISL_886620 EPI_ISL_886696 EPI_ISL_886713 EPI_ISL_886766 EPI_ISL_886786 EPI_ISL_886787 EPI_ISL_886826 EPI_ISL_886908 EPI_ISL_886958 EPI_ISL_887096 EPI_ISL_887699 EPI_ISL_888372 EPI_ISL_888468	Labcorp	Genomics and Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Peter W. Cook,Dhwani Batra,Ben L. Rambo-Martin,Summer Galloway,Brian Krueger,Minoo Agarwal,Eyad Almasri,Debbie Boles,Ayla Burns,Nuthawin Charoensri,Oren Cohen,Susan Countryman,Mary Ann Cristobal,Bobbi Croy,Suzanne Dale,Hrushikesh Deshmukh,Amanda Douglas,Vincent Drouillon,Marcia Eisenberg,Howard Engler,Rama Ghatti,Prashant Gupta,Susan Hicks,Jake Humphrey,Lax Iyer,Manoj Jain,Mohan Kolli,Tim Kuphal,Stanley Letovsky,Michael Levandoski,Craig Lukasik,Jonathan Meltzer,Brian Norvell,Mindy Nye,Scott Parker,Christos Petropoulos,John Pruitt,Steven Ragan,Scott Ryan,Mike Sapeta,Jana Schroth,Suresh Babu Selvaraju,Goran Stevovic,Amanda Suchanek,Andrea Throop,Lyndon Tilson,Thomas Urban,Joe Voshell,Kimberly Wagner,Jonathan Williams,Mary Williamson,Qian Zeng,Tricia Zwiefelhofer,Clinton R. Paden,Suxiang Tong,Duncan MacCannell,
EPI_ISL_903747 EPI_ISL_903774 EPI_ISL_903791 EPI_ISL_903817 EPI_ISL_903845 EPI_ISL_903885 EPI_ISL_903901	NE Public Health Laboratory	Genomics and Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Krista Queen, Yan Li, Ying Tao, Jing Zhang, Anna Uehara, Anna Montmayeur, Clinton R. Paden, Peter W. Cook, Rachel Marine, Mili Sheth, Jasmine Padilla, Sarah Nobles, Mark Burroughs, Lori Rowe, Haibin Wang, Ben L. Rambo-Martin, Dhwani Batra, Justin Lee, Suxiang Tong

EPI_ISL_428202 EPI_ISL_428203 EPI_ISL_428204 EPI_ISL_428205 EPI_ISL_428206 EPI_ISL_466652 EPI_ISL_466653 EPI_ISL_466654 EPI_ISL_466655 EPI_ISL_466656 EPI_ISL_466657 EPI_ISL_466658 EPI_ISL_466659 EPI_ISL_466660 EPI_ISL_466661 EPI_ISL_466662 EPI_ISL_466663 EPI_ISL_466664 EPI_ISL_466665 EPI_ISL_466666 EPI_ISL_466667 EPI_ISL_466668 EPI_ISL_466669 EPI_ISL_466670 EPI_ISL_466671 EPI_ISL_466672 EPI_ISL_466673 EPI_ISL_466674 EPI_ISL_466675 EPI_ISL_466676 EPI_ISL_466677 EPI_ISL_466678 EPI_ISL_466679 EPI_ISL_466680 EPI_ISL_466681 EPI_ISL_466682 EPI_ISL_466683 EPI_ISL_467298 EPI_ISL_475174 EPI_ISL_475175 EPI_ISL_475176 EPI_ISL_475177 EPI_ISL_475178 EPI_ISL_475179 EPI_ISL_475180 EPI_ISL_475181 EPI_ISL_475182 EPI_ISL_475183 EPI_ISL_475184 EPI_ISL_475185 EPI_ISL_475186 EPI_ISL_475187 EPI_ISL_475188 EPI_ISL_475189 EPI_ISL_475191 EPI_ISL_475192 EPI_ISL_475193 EPI_ISL_475194 EPI_ISL_475195 EPI_ISL_475196 EPI_ISL_475197 EPI_ISL_475198 EPI_ISL_475199 EPI_ISL_475200 EPI_ISL_475201 EPI_ISL_475202 EPI_ISL_475203 EPI_ISL_475204 EPI_ISL_475205 EPI_ISL_475206	Nebraska Public Health Laboratory	UNMC COVID-19 Response Team	UNMC COVID-19 Response Team

EPI_ISL_475207 EPI_ISL_475208 EPI_ISL_475209 EPI_ISL_475210 EPI_ISL_475211 EPI_ISL_475212 EPI_ISL_475213 EPI_ISL_475214 EPI_ISL_475215 EPI_ISL_475216 EPI_ISL_475217 EPI_ISL_475218 EPI_ISL_475219 EPI_ISL_475220 EPI_ISL_475221 EPI_ISL_475222 EPI_ISL_475223 EPI_ISL_475224 EPI_ISL_475225 EPI_ISL_475226 EPI_ISL_475227 EPI_ISL_475228 EPI_ISL_475229 EPI_ISL_475230 EPI_ISL_475231 EPI_ISL_475233 EPI_ISL_475234 EPI_ISL_475235 EPI_ISL_475236 EPI_ISL_475237 EPI_ISL_732820 EPI_ISL_732821 EPI_ISL_732822 EPI_ISL_732823 EPI_ISL_732824 EPI_ISL_732825 EPI_ISL_732826			

eTable 2. SARS-CoV-2 RNA Detection in School Building Air and Surface Samples

Pilot Week	Building Location	School A		School B		School C	
		Air	Surface	Air	Surface	Air	Surface
Week 1	Choir	**P**	N	**P**	**P**	N	N
	Band	N	N	N	N	N	N
	Hallway	N	N	--	--	--	--
	Bathroom	--	--	N	N	N	N
	Classroom	N	N	N	N	N	N
	Cafeteria	N	N	N	N	N	N
Week 2	Choir	N	N	N	N	N	N
	Band	N	N	N	N	N	N
	Hallway	--	--	--	--	--	--
	Bathroom	N	N	N	N	N	N
	Classroom	N	N	N	N	N	N
	Cafeteria	N	N	N	N	N	N
Week 4	Choir	N	N	N	N	N	N
	Band	N	N	N	N	N	N
	Hallway	N	N	N	N	N	N
	Bathroom	--	--	--	--	--	--
	Classroom	N	N	N	N	N	N
	Cafeteria	N	N	N	N	N	N
Week 5	Choir	N	N	N	N	N	N
	Band	N	N	N	N	N	N
	Hallway	N	N	N	N	N	N
	Bathroom	--	--	--	--	--	--
	Classroom	N	N	N	N	N	N
	Cafeteria	N	N	N	N	N	N

eTable 3. Demographic Risk Factors for SARS-CoV-2 Infection in Pilot Participants

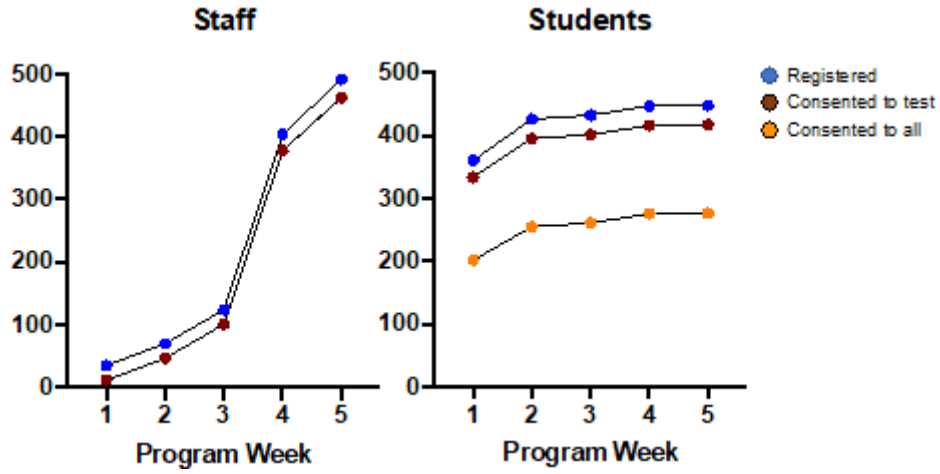
Demographics Among Students (unadjusted)[a]				
	Overall (315)	**Cases (22)**	**Control (293)**	**Association (p-value)[b]**
Band (yes)	62 (20)	0 (0)	62 (100)	0.9503
Choir (yes)	65 (21)	7 (32)	58 (20)	0.1851
School				
School A	139 (44)	15 (68)	124 (42)	**0.0191**
School B	71 (23)	1 (5)	70 (24)	
School C	105 (33)	6 (27)	99 (34)	
Grade				
5-6	77 (24)	4 (20)	73 (25)	0.3647
7-8	99 (31)	3 (14)	96 (33)	
9-10	71 (23)	7 (27)	64 (22)	
11-12	68 (22)	8 (36)	60 (20)	

Demographics Among Staff (unadjusted)				
	Overall (455)	**Cases (24)**	**Control (431)**	**Association (p-value)**
School				
School A	178 (39)	14 (58)	164 (38)	0.1528
School B	132 (29)	5 (21)	127 (29)	
School C	145 (32)	5 (21)	140 (32)	
Position				
Teacher	243 (53)	12 (50)	231 (54)	0.9005
Administration	58 (13)	3 (13)	55 (13)	
Cafeteria	29 (6)	0 (0)	28 (7)	
Custodian/Security	14 (3)	2 (8)	12 (3)	
Physical Education	20 (4)	1 (4)	19 (4)	
Special Ed/Para	75 (16)	5 (21)	70 (16)	
Student Support	17 (4)	1 (4)	16 (4)	

[a]Cell values reflect "N (column percent)"

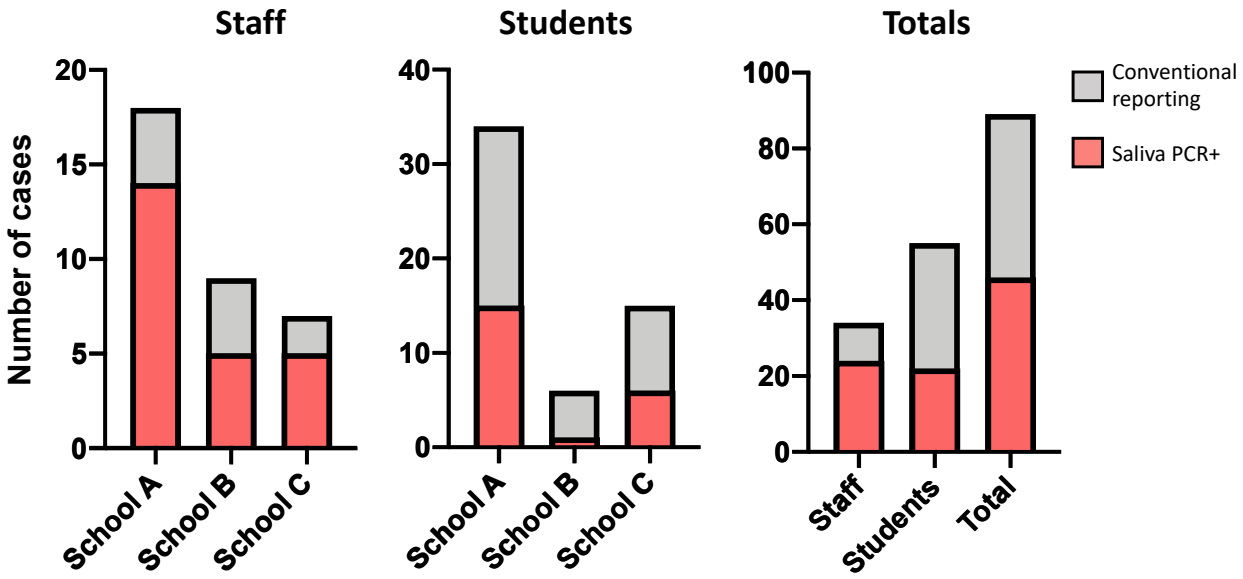
[b]p-values calculated using type 3 analysis of effects

eFigure 1. Cumulative Registrations and Consents Over the Pilot Program Period.
Consent for testing as well as consent for treatment and ancillary clinical services was required for program participation. Student participation was limited by a high number of declinations for consent to treatment and/or ancillary clinical services.

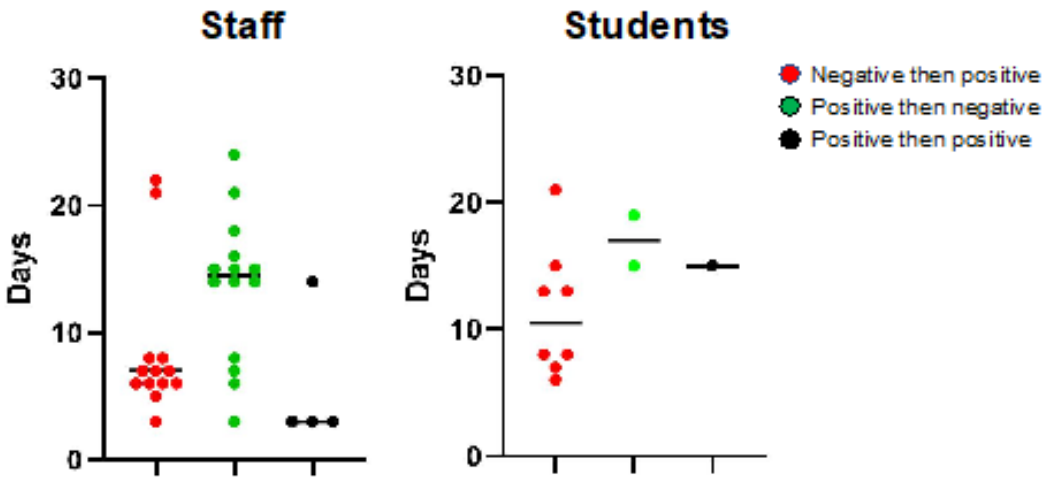


eFigure 2. Incremental Surveillance Value of Asymptomatic Saliva Testing in the Pilot Program.

Number of cases identified (and so persons representing transmission risk and removed from in school activities) through the pilot in contrast to those identified by usual passive case capture.



eFigure 3. Time Interval for SARS-CoV-2 Saliva PCR Test Conversions



eFigure 4. Weekly SARS-CoV-2 Case Detection by Saliva PCR Among Choir Students in Each Pilot School.
Number of cases detected in each week is denoted in the circles.